Rev. Soc. Mex. Hist. Nat., 47:29-40 (1997).

Some Observations on the Fine Structure of Trophozoite and Giant form of the Salt Marsh Heterotrich Ciliate *Fabrea salina* (Henneguy).*

Algunas observaciones sobre la estructura fina de trofozoides y la forma gigante del heterotrico salobre, Fabrea salina (Henneguy).

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ABSTRACT

Surface and internal organelles of the trophozoite and giant forms of the marine heterotrich ciliate *Fabrea salina* were studied by Nomarski interference contrast microscopy and by scanning and transmission electron microscopy. The AZM starts at the apical tip and extends halfway downward along the left ventral surface where it spirals right and descends into the cell terminating at the cytostome. Each AM consists of rows of dikinetids except near the posterior end where there are three rows of kinetids. Adoral kinetids are linked by crisscross fibrous connections. A non-ciliated border, with subpellicular microtubule, separates the oral groove from the AZM. The oral groove is ciliated from the apical tip to the vestibulum. There is no undulating membrane on the right side of the oral groove. Somatic ciliature, consisting of dikinetids, covers the entire cell and occurs in rows between pellicular ridges. Alveolar membranes and an epiplasm are located under the cell membrane. Mucocysts and some pigment granules were noted in the epiplasm along with Golgi apparatus, mitochondria and clear vacuoles. Numerous storage granules are also present. A membrane bound reticulated cisternal structure, possibly the remains of the exhausted food vacuole, was found in the vicinity of the cytoproct. The filiform macronucleus contained numerous nucleoli. Micronuclei are located close to the macronucleus. Giant forms differ from the vegetative form in the positioning of the buccal apparatus as well as in the degree of vacuolization.

Key words: Electron microscopy; *Fabrea salina*. Nomarski interference microscopy; somatic and buccal ultrastructure.

RESUMEN

Los organoides superficiales e internos de lo trofozoides y la forma gigante del ciliado heterotrico *Fabrea salina* fueron estudiados con microscopía de contraste de interferencia Normaskiy por microscopía electrónica de barrido y transmisión. Las ZAM comienzan desde la punta apical y se extienden hasta la mitad de la superfice ventral izquierda donde gira a la derecha para descender dentro de la célula terminando en el citostoma. Cada MA se compone de columnas de diquinétidas excepto cerca del límite posterior en donde se conforma de tres columnas de cinetidas. Las cinetidas adorales están unidos por conecciones fibrosas entrecruzadas. El borde no ciliar, consta de un microtúbulo subpellicular, que separa al surco oral de la ZAM. El surco oral es ciliado desde la punta apical hasta el vestíbulo. Ahí, en el lado derecho del canal oral la membrana no es ondulada. La ciliatura somática, consiste de diquinetidas, que cubren toda la celula y se distribuyen en columnas entre las crestas peliculares. Las membranas alveolares y un epiplasma son localizados debajo de la membrana celular. Mucocistos y algunos gránulos de pigmentos se observaron en el epiplasma y a

* Enviado para el Volumen No. 47 de la RSMHN, dedicado al Dr. Eucario López-Ochoterena.

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lo largo del aparato de Golgi, mitocondias y vacuolas claras. Además, se presentan numerosos gránulos de almacenamiento. Una membrana reticular rodea a una estructura cisternal, posiblemente las reminiscencias de las vacuolas alimenticias, se encuentran próximas a el citoprocto. Los macronúcleos filiformes contienen numerosos nucleolos. Los micronúcleos se localizan rodeados por los macronúcleos. Las formas gigantes difieren de la forma vegetativa por la posición del aparato bucal así como en el grado de vacuolización.

Palabras clave: Microscopía electrónica, *Fabrea salina*, microscopía de interferencia Nomarski, Ultraestructura somática y bucal.

Introduction

Fabrea salina, a euryhaline heterotrich ciliate, was first describes by Stepanow (1885, 1886) as a species of *Climacostomum* found in salt lakes of Slaviansk. Shortly thereafter Henneguy (1890), noting that the ciliate did not fulfill the definition of a species of *Climacostomum*, renamed it. Repak (1972) placed the organism within a new family Climacostomaidae. The morphology of *F. salina* as revealed with bright field microscopy has been studied by a number of investigators (Ellis, 1937; Entz, 1904; Fauré-Fremiet, 1911, 1912; (Kirby, 1934; Tuffrau, 1967; Villeneuve-Brachon, 1940).

Several heterotrich ciliates have been studied with electron microscopy these include: *Blepharisma* (Dembitzer & Hirshfield, 1966; Inaba, *et al*, 1958; Kennedy, 1965), *Climacostomum* (Peck *et al*, 1975), *Condylostoma* (Bohatier, 1978; Yagiu & Shigenaka, 1958a, 1958b), *Eufolliculina* (Mulisch & Hausmann, 1984), *Nyctotherus* (King *et al*, 1961; Puytorac & Otkem, 1967), *Phacodinium* (Dider & Dragesco, 1979), *Plagiotoma* (Albaret, 1973), *Sicuophora* (Puytorac & Grain, 1968), *Spirostomum* (Finley, 1951, 1955; Grain, 1968; Inaba, 1960; Randall, 1957), *Stentor* (Inaba, 1959; Pelvat, 1985; Randall & Jackson, 1958). To date there is no literature on the fine structure of *Fabrea salina* in trophic or giant form. Giant protozoa were reported by several investigators earlier in this century in several protozoa species ranging from flagellates to rhizopods and ciliates (Giese, 1973). Giant forms of *Blepharisma* species are perhaps the most widely studied (Bhandary & Hirshfield, 1964; Giese, 1973; Kasturi Bair & Dilli, 1976; Lennartz & Bovee, 1980; Nilsson, 1967; Pierce *et al*, 1978; Rickards & Lynn, 1985). Giant *Blepharisma* are considerably larger (1.5-2X) than the trophozoite. The body width, number of adoral membranelles (AM), adoral zone of membranelles (AZM) length, number of somatic ciliary rows (SCR) also increase in the same fashion. Furthermore the giants are usually capable of cannibalism (Pierce *et al*, 1978). The objective of this investigation is to study the fine structure of the trophozoite and giant *F. salina* through the use of transmission and scanning electron microscopy.

Materials and Methods

Stocks were grown in screw-capped test tubes containing 10 ml of sterile, seawater at pH 8, 26 °/oo salinity that had been filtered with activated charcoal, 2 barley kernels and *Escherichia coli* (ornithine positive; rabnose negative) *F. salina* was obtained from the A. Kubalski (Dept. Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland). Cultures were kept at 26°C and subcultured monthly. Dixenic cultures were prepared and maintained as previously described by Repak [37, 38]. Some cultures were prepared using Sea Salts at 66 ‰. Photomicrography was by a Leitz Nomarsky Interference Microscopy and an Orthomat automatic camera.

Samples for transmission electron microscopy were fixed for 1 h in 5% (v/v) glutaraldehyde 25°C prepared in 0.25M sucrose/0.1 M cacodyla buffer at pH 7.2. Following aspiration of the fixative, the specimens were washed in cold (5- 10°C) buffer solution for 15 to 20 min. The fixed cells were then post-fixed for 2 h at 3°C in 2% (w/v) osmium tetroxide prepared in the same cacodylate buffer used for the glutaraldehyde fixation, rinsed with water and dehydrated through a series of aqueous acetone solutions. The ciliates were infiltrated and embedded with Epon 812 and sectioned with a diamond knife, using a Porter-Blum MT2 ultramicrotome. Sections were collected on uncoated copper grids, post-stained with alkaline lead citrate, and viewed with a Philips EM 201 electron microscope operated at 60 or 80 kV.

Specimens for scanning electron microscopy were fixed with Kamovsky's mixture (Ito & Kamovsky, 1968; Kamovsky, 1965) washed with distilled water, suspended in 10% (v/v) aqueous ethanol solution, deposited onto aluminum stubs coated with double-sided sticky tape freeze dried using liquid nitrogen, and coated with gold pallodium for viewing with a Cambridge Stereoscan 250 Mk 2 scanning electron microscope.

Results

Scanning, Electron Microscopy

The organism is pyriform in general shape (Figs. 1-4). The buccal apparatus begins at the apical tip proboscis and extends posteriorly along the ventral surface to about l/2 the length of the ciliate. It consists of an AZM on the organism's left, a ciliated buccal groove just right of the AZM and a cytostome. The AZM begins at the apical tip, runs the length of the buccal apparatus up to the l/2way point then turns right across the ventral surface. On the left side it spirals inward and downward into the cytoplasm (Fig. 2).

The AZM is embedded in its own channel, that is bordered centripetally by a pellicular ridge which lacks cilia. Medial to the AZM is a ciliated buccal groove (Fig 2 & 4). The portion of the ventral surface bordering upon the buccal groove is part of the somatic ciliature. This area is ciliated, elevated and rounded (Fig. 2). There is no evidence of an undulating membrane. The somatic ciliature is located within pellicular grooves (Figs.3 & 6).

Figs. 6 & 7 show the details of giant forms of *F. salina*. The buccal apparatus is arranged more in a Stentorian fashion than that of the trophozoite. The buccal apparatus has become planar and oriented anteriorly rather than ventrally. The apical tip, proboscis, is not as prolonged as that of the trophozoite and resembles a nipple. The body of the giant is generally spherical compared to the trophozoite.

Transmission Electron Microscopy

TROPHOZOITE

The AZM begins at the anterior tip of the trophic organism and is composed of two rows of numerous dikinetids. Each double row is separated from another set by pellicular ridges (Figs. 8, 9 and 2l). The remainder of the surface is composed of widely spaced individual somatic dikinetids in grooves forrned by pellicular ridges. The somatic ciliature is arranged in rows parallel to the longitudinal axis of the organism. Details of the somatic dikinetid arrangement (Fig. 22).

The surface of the organism is covered by a typical plasma membrane. Underlying the plasma membrane is a definitive alveolar layer (Fig. 8).

Details of the subpellicular to surface structure can be seen in Figs. 10 & 11. The oral polykinetids are arranged in a staggered order comparable to the hexagonal-packed polykinetids. However, aside from the staggered arrangement, the subpellicular fibrillar organization fits that describes for square-packed polykinetids (Fig. 11 & 2l). Closer to the cytostome region, the number of rows increases to 3 just prior to the termination of the AZM (Fig. 12 - 14). The AZM is anchored in a vacuolated subpellicular layer. The cilia of the AZM literally cover the space leading into the cytostome (Figs. 12 & 13). The membrane near the cytostome is typically lined with rows of perpendicularly oriented microtubule associated with small vacuoles (Fig. 15 & 16). The barren region is devoid of cilia. A singular layer of microtubule has been observed below the alveolar stratum (Fig. 17). The alveolar system in *Fabrea* is of interest because of its association with sheets of subpellicular microtubule in the medial portion of the AZM (Fig. 17). There appears a hint of possible function for such an alveolar system in this marine ciliate. It was noted that in ciliates taken from high salinity cultures >40 o/oo), the alveoli were very well developed as compared to organisms raised in lower salinities.

The transverse ribbon of microtubule extends tangentially and posteriorly. The postciliary ribbon of microtubule diverges from the posterior kinetosome at an angle of about 45° to the kinetodesmata. Each somatic postciliary ribbon connects to the distal portion of the kinetodesmata of the next dikinetid. The posterior ciliary ribbons of oral dikinetids, on the other hand, appear to be parallel to the kinetodesmata and then connect with them.

In the oral apparatus of *F. salina,* each member of a dikinetid pair are cross connected to each of the nearest four kinetosomes (Fig. 21). Large bands

of linear arranged fibrous material connect each medial kinetosome in a row. The kinetosomes connected to kinetodesmata are similarly connected by fibrils which appear to cross from the eighth triplet of the anterior organelle to the sixth triplet of the next posterior one. Another set of fibrils goes from the first anterior triplet to the sixth set. The two rows are interconnected by two or three fibrils extending from a more lateral kinetosome at an angle of 45° posteriorly to triplets seven and eight. From each kinetosome one fibril extends from triple 3 anteriorly to triplet 7. Parasomal sacs are dispersed in between the oral kinetids. The nematodesmata are medially located deep below the oral apparatus, (Fig. 10 and 11).

In the somatic regions, Golgi are singularly scattered in the ectoplasm. They appear as thin stacks of flattened cisternae surrounded by mitochondria (Fig. 18). In addition, numerous mucocysts and granules are present.

A membrane-bound reticulated cisternal structure, not much larger than the surrounding mitochondria, was found in the posterior portion of the cell just above the cytoproct. Dense fibrous material fills the cisternae of this organelle (Fig. 19).

The endoplasm contains numerous mitochondria, vacuoles of varying sizes and what appears to be polysaccharide storage granules. There were large food vacuoles containing either bacteria or algae, depending upon the diet.

The macronucleus is filiform. The periphery of the macronucleus appears to have an undulate appearance. Numerous nucleoli are evident (Fig. 20). Micronuclei were found close to the macronucleus. The location of the micronuclei, close to that of the macronucleus, made them difficult to visualize using conventional cytological staining techniques, e.g. Feulgen. One interesting observation is that the nuclear material does not respond well to Feulgen reagents or the use of DAPI.

GIANTS

Giants are nearly double in size compared to the trophozoite. Another difference is that there is an increase in vacuolation of the cytoplasm of the giant. Both forms have polykinetids with 2 or 3 rows in the AZM. The one notable distinction is the alteration in the shape of the mouth and the shrinkage of the anterior end. A great deal of vacuolization is commonly observed in giant *Fabrea*.

Discussion

That *Fabrea salina* is a heterotrichous ciliate is evident from the dikinetid pattern of its oral and somatic ciliature (Lynn, 1981). Although the dikinetid patterns of *F. salina* are similar to those of other heterotrich ciliates, there is sufficient variability accountable as species differences. In *F. salina* the kinetodesmatal fibrils are rather outstanding as compared to those of *C. virens* (Peck *et al*, 1975) and laterally directed. These kinds of connections are not as evident in *C. virens*. Similar interconnections were noted for *Eufolliculina uhligi* (Mulisch & Hausmann 1984).

Perhaps there is a relationship between water conservation and the expansion of the alveoli but we have no definitive evidence for the relationship.

A membrane bound reticulated cisternal-like structure (Fig. 19) found in the posterior of *F. salina* may represent the remains of an exocytized food vacuole in the process of being recycled; especially since in one section a cytoproct was located nearby. Similar structures have been describes in *Paramecium* (Fok & Allen, 1988).

Giant *Fabrea* were initially overlooked as a stage in the life cycle of this heterotrich. Henneguy [15] noted that some *Fabrea* achieved length and widths of 450 μm X 221 μm. Entz (1904) estimated some forms reach a size of 600 μm X 300 μm. Kirby's (1934) upper limits were 221 μm X 125 μm. Ellis (1904) was the first to note the existence of giants. The normal range is 120-180 mm in length and 67-80 mm in width. The giant form also misled some investigators (Kahl, 1935) into thinking that *Fabrea* belonged among the Stentoridae because of the resemblance of the buccal apparatus to that of *Stentor*. Pierce *et al.* (1978) suggested that in *Blepharisma* an increase in water retention may be responsible for inhibiting cytokinesis and forcing the utilization of materials from a "membrane bank". Lennartz & Bovee (1980) found that α-tocopherol succinate at about 10^{-5} M affects the membranes of the trophozoites in *Blepharisma*

causing giant formation. Attempts at using Vitamin E on *Fabrea* so far have failed to produce giants. This might be a problem related to the salt content of the marine environment. More biometrical and cytological studies of the details of giant forms are needed.

According to Corliss (1961), heterotrichs characteristically possess uniform somatic ciliature and conspicuous buccal ciliature with an adoral (oral) zone of many membranelles. Heterotrichs may have arisen from hymenostomes, possessing a tetrahymena mouth pattern, which consists of an undulating membrane on the organism's right and a tripartite AZM on the left. Corliss' (1961) scheme does not consider the evolution among the heterotrichs. Jankowski (1964) presented a scheme giving details of the possible evolution among the heterotrichs and suggested a prototype organism, *Blepharisma*.

All heterotrichs possess the elaborate AZM but differ from each other and the tetrahymenal pattern. *Stentor* lacks an undulating membrane in its apically arranged circular buccal apparatus, *Condylostoma* possess an open-ended shovel-shaped buccal apparatus instead of pyriform one. *Spirostomum* and *Parablepharisma* possess reduced or greatly exaggerated undulating membranes. *Metopus* has an overlapped AZM and buccal apparatus similar to that of *Blepharisma*. *Fabrea* has a pyriform body shape similar to that of *Blepharisma*. The mouth of *Fabrea* is also pyrifonn. It was Jankowski's (1964) contention that the buccal pattern of *Blepharisma* may be the prototype for other heterotrichs.

There are other features that correlated the relationship among heterotrichs. Many of these ciliates are pigmented. Most *Blepharisma* species are noted for their purple-reddish pigment, zoopurpurin. Some *Blepharisma* are pinkish or devoid of pigment. Some *Stentor* species possess stentorin which varies from blue green to alizarin red depending on the kind of lighting (Tarter, 1961). The purplish-red of *S. igneus* may be the same as the zoopurpurin of *Blepharisma*. *S. niger* has a brown pigment as may does a variety of *S. igneus* nigricans. The Folliculinids, e.g. *Eufolliculina uhligi*, (Mulisch *et al*, 1984) are blue in color. *Fabrea* is colored brownish in most cultures. However, when forming cysts, the pigment of the early cyst stages before wall formation is a deep purple color. Pigment may serve as a protective devise against predation or may enable these organisms to orient themselves to light or protect themselves against ultraviolet light (Giese, 1973).

Giant formation is shared at least by two genera, *Blepharisma* and *Fabrea*. It might be that some species are just too big to become giants, e.g. *Stentor* and *Spirostomum*.

At the ultrastructural level the picture is a bit more confusing. In *Blepharisma* each adorar membranelle according to Kennedy consists of a plate of three rows of basal granules arranged in a hexagonal pattern. Fibrils are joined to form a fan shaped structure that tapers toward the middle of the buccal apparatus and forks connecting to neighboring membranelles. Fibrils also cross to the undulating membrane. The vestibulum of the buccal structure in *Blepharisma* is devoid of cilia. *Fabrea* has an oral ridge to which the adoral membranelle appear to be connected. The vestibulum is covered with cilia and there is no undulating membrane. Most of the AZM of *Fabrea* consist of two rows of cilia except near the cytostome where the number of rows increases to three per membranelle. Lynn (1991) argues for diversity among the heterotrich kinetids. Lynn's characterization of a heterotrich dikinetid is "a ciliated anterior kinetosome with a curved transverse ribbon; a posterior kinetosome, ciliated in the rear of the cell, with transverse ribbon of 5-6 microtubules at triplets 4 and 5, a divergent postciliary ribbon of about 15 microtubules that extends posteriorly to form a postciliodesma sensu stricto, a laterally-directed non-striated kinetodesmal fibril at triplets 5, and 6 and a dense fibril near triplets 3 & 4 that extends laterally left." According to Lynn (1991), *Eufolliculina*, *Condylostoma*, *Stentor* and *Climacostomum* constitute one grouping of heterotrich which share this pattern of dikinetid. *Sicuophora's* dikinetid differs from those of this group as well as from that of *Transitella*. There are then 3 groupings depending upon the dikinetid pattern. *Fabrea* as well as that of *Blepharisma* appears to fit in the grouping along with *Stentor* etc. Where does *Spirostomum* fit in? I would guess that it will join that of the *Stentor*, etc. grouping. There is also the question of *Nytotherus* and the remaining heterotrichs as to their relationship to the other heterotrichs. A study of their kinetid pattern is in order.

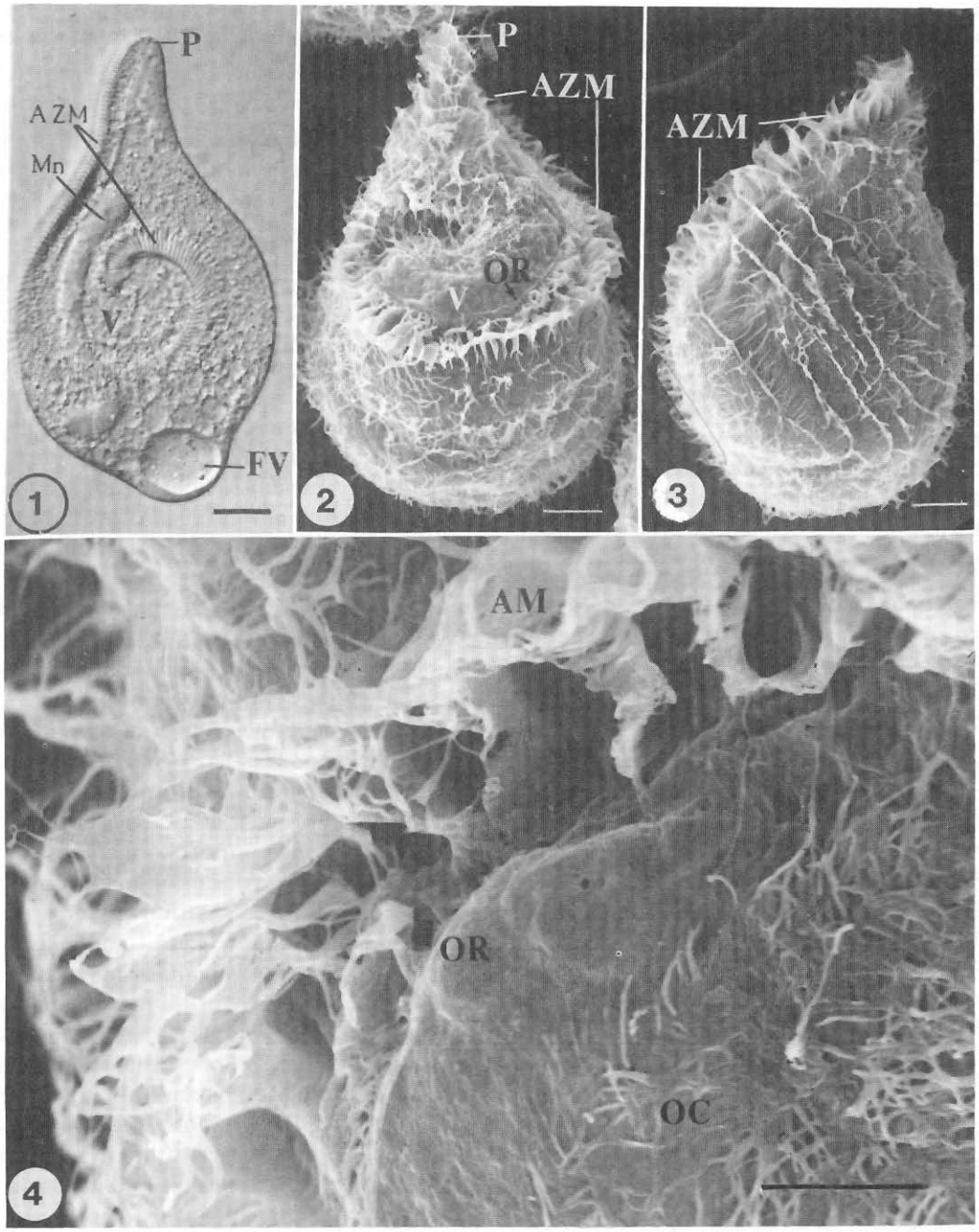
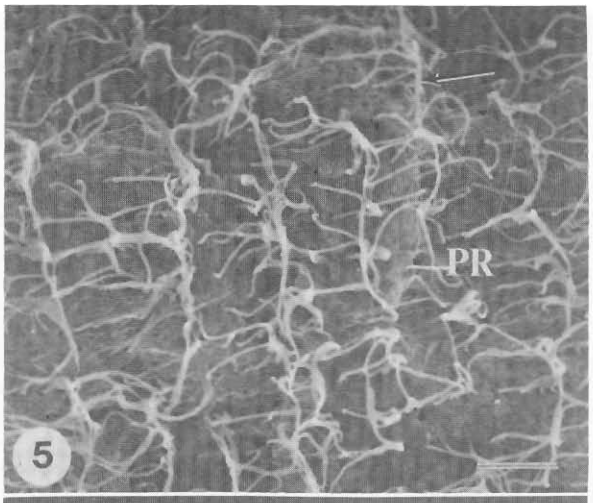


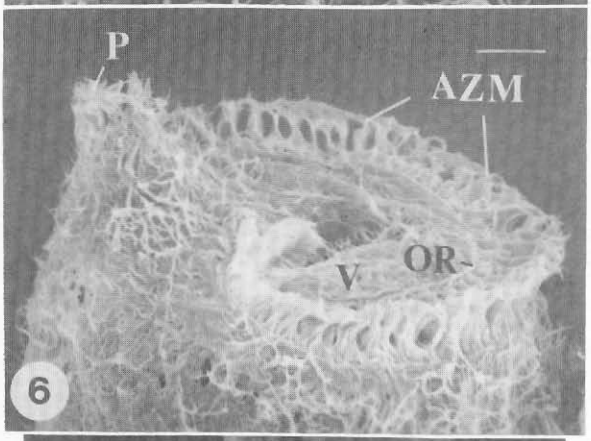
Fig. 1. Nomarski interference phase microscopy of the trophozoite of *F. salina*. N.B. The left side of the organism is on the viewer's left. Photomicrography taken through the dorsal surface. Note the proboscis or anterior tip, the adoral zone of membranelle, vestibular region of the oral groove, filiform macronucleus and the food vacuole shortly before exocytosis. Bar scale = 20 μm; AZM = adoral zone of membranelles; Fv = food vacuole; Mn = macronucleus; P = proboscis.

Fig. 2. Ventral SEM view of the trophozoite of *Esalina*. Same orientation as the previous photomicrograph and showing the prominent oral pellicular ridge. Bar scale =30 μm; AZM = adoral zone of membranelles; P = proboscis.

Fig. 3. Left SEM view of the trophozoite. Note metachromatic waves of somatic cilia. Bar scale = 30 μm; AZM = adoral zone of membranelles.

Fig. 4. SEM view of a portion of the AZM showing the channel in which it is embedded, the individual adoral membranelle, oral cilia and the oral ridge. Bar scale = 10 μm; AM = adoral membranelle; OC = oral cilia; OR = oral ridge.





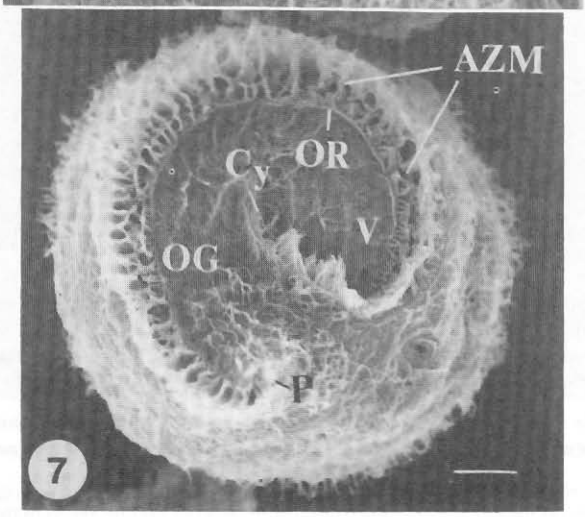


Fig. 5. SEM view of somatic cilia located on the surface of the organism within pellicular groves and separated by pellicular ridges. Bar scale = 10 μm; PR = pellicular ridge.

Fig. 6. SEM lateral view of the giant form of *F. salina*. Note the stentor-like arrange of the buccal apparatus. The proboscis is to the left of the photomirograph. Bar scale = 29 mm; AZM = adoral zone of membranelles; OR = oral ridge; P = proboscis; V = vestibulum.

Fig. 7. Apical view of the giant form showing the AZM showing the proboscis, oral groove, cytostome, oral ridge, Vestibulum and AZM. Bar scale = 27 μm; AZM = adoral zone of membranelles; Cy = cytostome; OG = oral groove; OR = oral ridge.

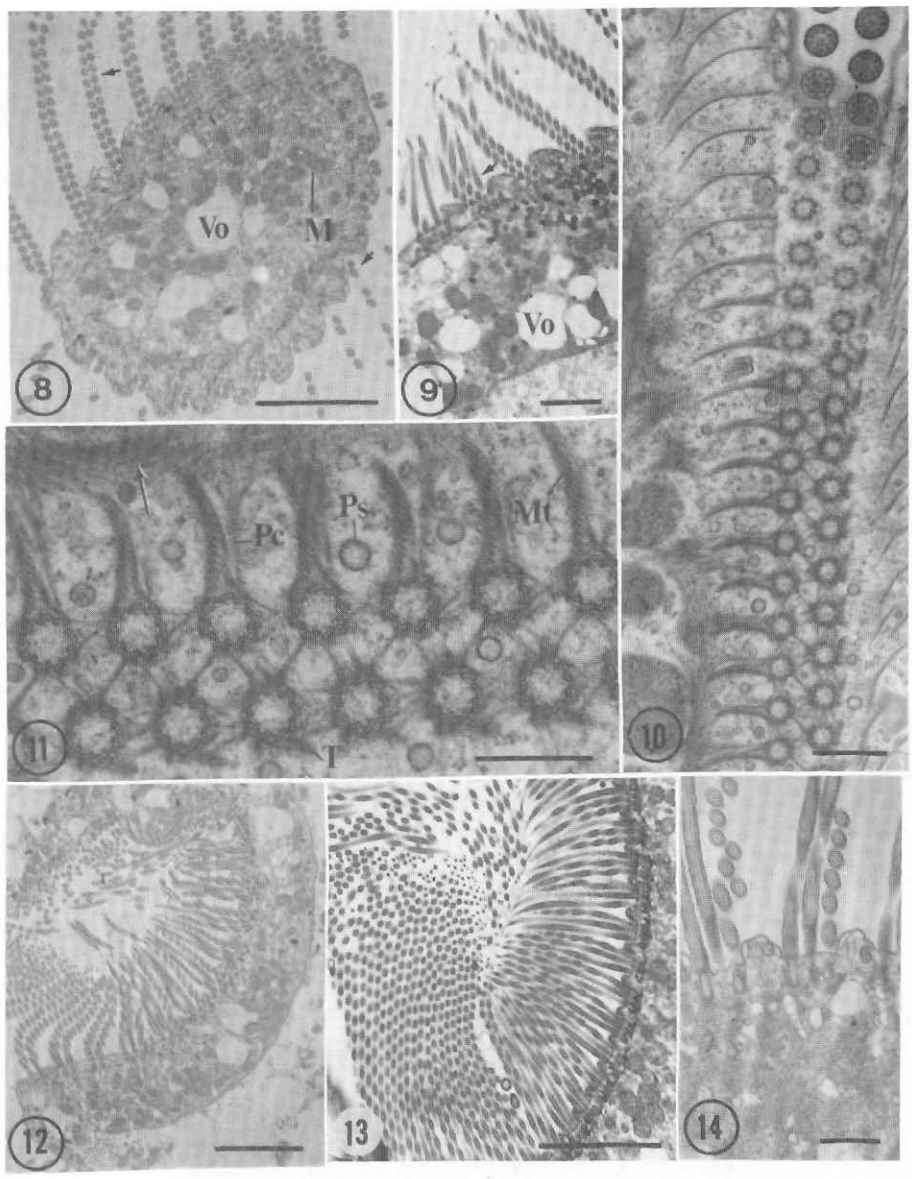


Fig. 8. Apical cross section of a trophozoite showing the adoral membranelles at the top arrow. Somatic ciliature (arrow) in their pellicular grooves and separated by pellicular ridges at the bottom of the photomicrograph. Vacuoles are visible in the endoplasm. Especially notable are the numbers of mitochondria in this region. The alveolar region of the pellicle shows vacuolization. Dikinetids are also evident in the ectoplasm. Bar scale = 5 μm; Vo = vacuole.

Fig. 9. Apical oblique section of the trophozoite taken from the ventral region of the buccal apparatus of a giant. N.B. the increase from two to three ciliary rows (arrow); and the increased number of vacuoles in the ectoplasmic and endoplasmic region. Bar scale = 2.5 μm; Vo = vacuole.

Fig. 10. Oblique slice through an adoral membranelle of the trophozoite showing various levels of the cilia, kinetosomal and dikinetid connections. Bar scale = 0.5 μm; Pc = postciliary ribbon of microtubules; Ps = parasomal sac; Mt = microtubules.

Fig. 11. Enlargement of the basal structure of the AZM showing the interconnecting dikinetids, associated parasomal sacs and kinetodesmata. Bar scale = 0.5 μm.

Fig. 12. Oral ciliature in the areas leading to the cytostome of a trophozoite. Bar scale = 5 μm.

Fig. 13. Cytostomal regional showing the spiral and AM rows. Bar scale = 5 μm

Fig. 14. Higher magnification of the rows with 3 cilia across from the buccal apparatus of a giant. Bar scale = 1 μm.

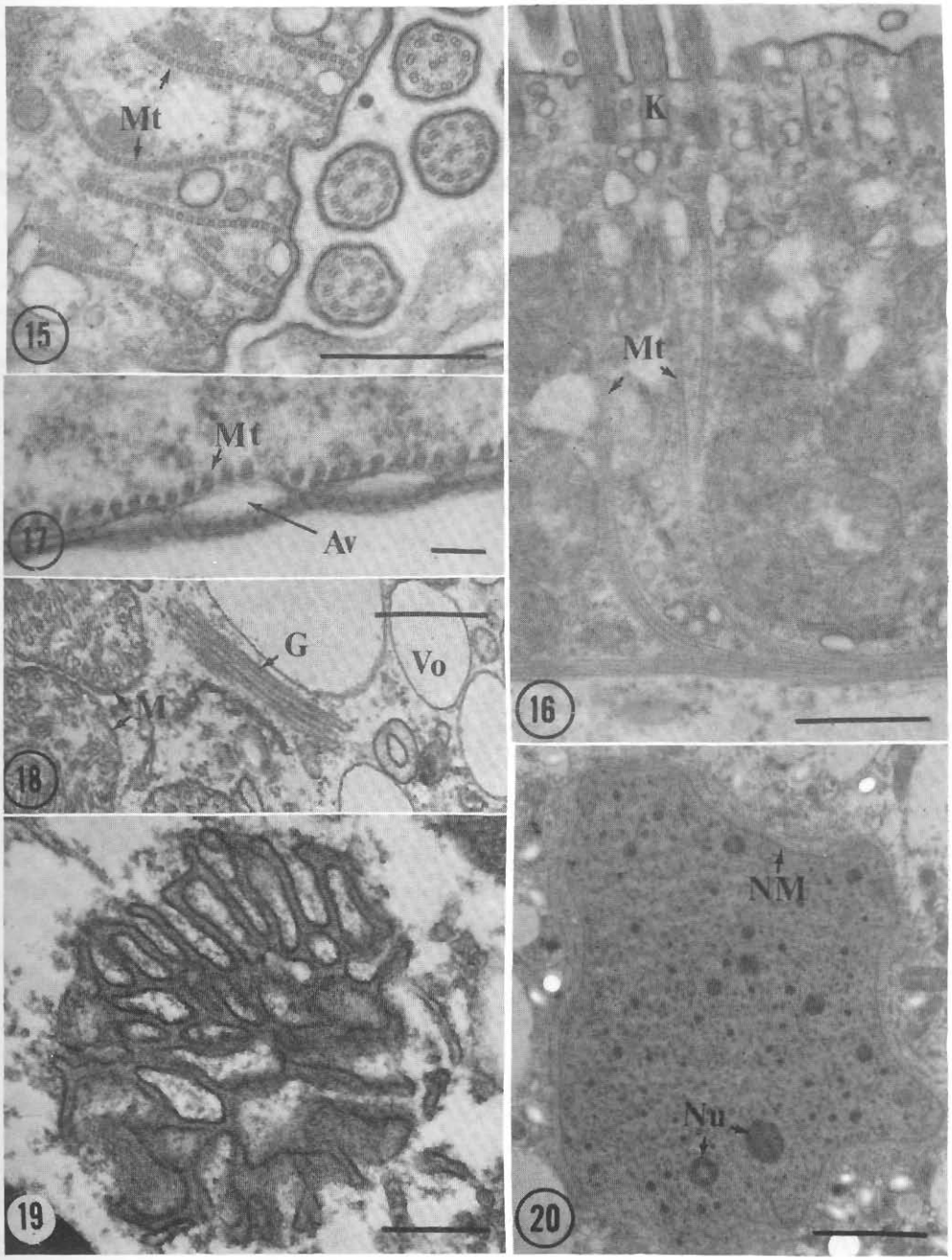


Fig. 15. A view of the buccal groove showing the underlying microtubular sheets. Bar scale = 0.5 μm; Mt = microtubules.

Fig. 16. Microtubular roots of oral kinetids including clearly visible kinetosomes. Bar scale = 1 μm; K = kinetosome; Mt = microtubules.

Fig. 17. Barren area showing microtubular elements originating from the adoral membranelles underlying the alveolar region of the pellicle. Bar scale = 0.2 μm; Av = aveolar region; Mt = microtubules.

Fig. 18. Golgi apparatus in the somatic areas of the ectoplasm, surrounded by mitochondria and vacuoles. Bar scale = 0.5 μm; G = Golgi apparatus; Vo = vacuole.

Fig. 19. Reticulated body in the posterior of the cell. Bar scale = 0. 5 μm.

Fig.20 Cross section of the macronucleus showing nucleoli and nuclear membrane. Bar scale = 5 μm.

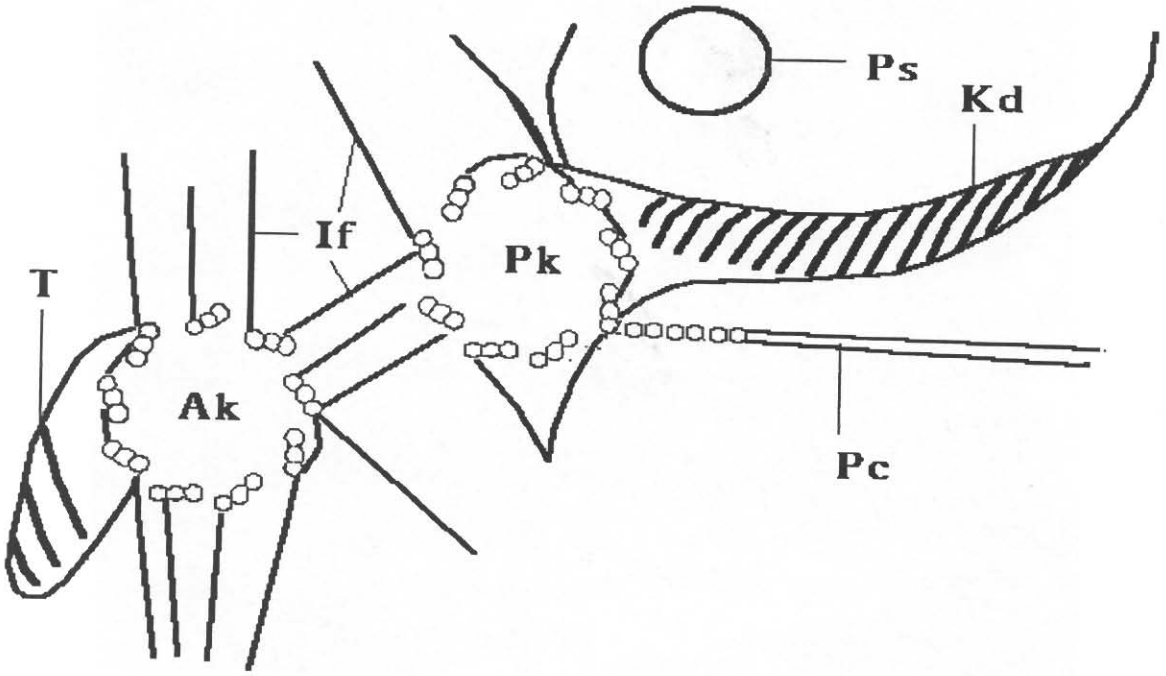


Fig. 21. Diagrammatic representation of the dikinetid arrangement in the adoral membranelle. Ak = anterior kinetid; If = interconnecting fibrils; Kd = kinetodesmata; Pc = postciliary ribbon of microtubules; Pk = posterior kinetid; Ps = parasomal vacuole.; T = transverse ribbon of microtubules.

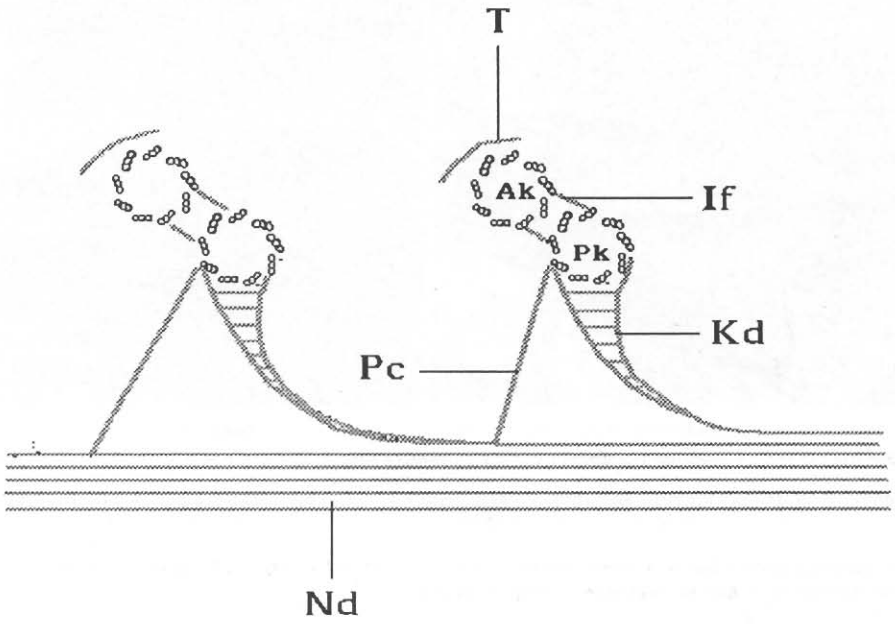


Fig. 22. Diagrammatic representation of somatic dikinetids and nematodesmata. Ak = anterior kinetid; If = interconnecting fibrils; Kd = kinetodesmata; Nd = nematodesmata; Pc = postciliary ribbon of microtubules; Pk = posterior kinetid; T = transverse ribbon of microtubules.

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